A137

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 35/16, 35/26, 37/04
C12N 5/02

A1

(11) International Publication Number: WO 92/05793

(43) International Publication Date: 16 April 1992 (16.04.92)

(21) International Application Number: PCT/US91/07283

(22) International Filing Date: 4 October 1991 (04.10.91)

(30) Priority data:
593,083 5 October 1990 (05.10.90) US

(71) Applicant *(for all designated States except US)*: MEDAREX, INC. [US/US]; 22 Commerce Drive, West Lebanon, NH 03784 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ROMET-LEMONNE, Jean-Loup [FR/FR]; 46, rue Vatonne, F-91190 Gif-Yvette (FR). FANGER, Michael, W. [US/US]; West View Lane, Box 421, Lebanon, NH 03766 (US).

(74) Agents: DECONTI, Giulio, A., Jr. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TARGETED IMMUNOSTIMULATION WITH BISPECIFIC REAGENTS

(57) Abstract

Immune response against an antigen is stimulated by administering the antigen in conjunction with a binding agent specific for an antigen-presenting cell such as a macrophage. The binding agent specifically binds a receptor of the antigen-presenting cell, such as an FC receptor, without being blocked by the endogenous ligand for the receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU**+	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE*	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

+ **Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.**

TARGETED IMMUNOSTIMULATION WITH BISPECIFIC REAGENTS

Background

Antigen molecules are recognized by the immune system after internal processing by antigen-presenting cells, generally mononuclear phagocytic cells such as macrophages. In order to present a proteinaceous antigen, the antigen-presenting cell first internalizes the antigen which is then broken down into small peptidic fragments by enzymes contained in vesicles in the cytoplasm of the antigen-presenting cells. After fragmentation, the peptides are linked to cellular major histocompatibility complex (MHC) molecules and presented on the presenting cell's surface to the immune system. Peptides presented in this way are recognized by the T-cell receptor which engages T-lymphocytes into the immune response against this antigen. This antigen presentation also stimulates the B lymphocytes for specific antibody production.

Complexes of antibody and antigen have been used to stimulate an immune response against the antigen. Antigen uptake through antigen-antibody conjugates bound to FcγR increases the efficiency of antigen presentation and thereby antigen-specific T-cell activation by human and mouse macrophages. Celis, E. and Chang, T.W. (1984) Science 224:297-299; Chang,

T.W. (1985) Immunol. Today 6:245-259; Manca, R. et al. (1988) Immunol. 140:2893-2898; Schalke, B.C.G. et al. (1985) J. Immunol. 134:3643; and Snider, D.P. and Segal, D.M. (1987) J. Immunol. 139:1053-1059. The binding of these complexes to FcγR is mediated by the Fc region of the antibody. This binding is susceptible to inhibition by physiological level of IgG.

Summary of the Invention

This invention pertains to a method of stimulating the immune response to an antigen by administering the antigen in conjunction with a binding agent which binds a surface receptor of an antigen-presenting cell without being blocked by natural ligand for the receptor and targets the antigen to the antigen-presenting cell.

In one embodiment, a bispecific binding agent is employed to target the antigen. The bispecific binding reagent has a binding specificity for the antigen and a binding specificity for a surface receptor of an antigen-presenting cell, such as a mononuclear phagocyte (e.g., a macrophage). The bispecific binding agent binds the cellular receptor, such as an Fc receptor, and targets the antigen, without substantially being blocked by the natural ligand for the receptor. In a preferred embodiment, the bispecific binding agent specifically binds the Fc receptor of an antigen-presenting cell for immunoglobulin G (IgG) without being blocked by IgG. In a particularly preferred embodiment, the agent specifically binds the high affinity Fc receptor for immunoglobulin G (FcγRI).

The bispecific binding agent can be a bispecific antibody or heteroantibody. The antigen to be targeted can be derived from a foreign pathogen or it can be derived from endogenous diseased host cells such as tumor cells. Generally, the antigen is administered as a preformed complex with the bispecific reagent. In some cases, however, the antigen and the bispecific binding agent may be administered separately or the bispecific binding agent may be administered alone.

In another embodiment of the invention, the antigen is directly bound to a receptor-binding agent to create bispecific molecules. For example, the antigen can be covalently coupled to an antibody which binds the Fc receptor without being blocked by IgG.

The method and compositions of this invention can be used to treat or prevent infectious diseases, to neutralize the acute phase of an infection by a pathogenic organism, to stimulate the immune system in instances of chronic infection of such an organism and to treat tumors.

Brief Description of the Figure

Figure 1 illustrates the enhanced antigen presentation by directing antigen to human FcγR.

Detailed Description of the Invention

In the method of this invention, an antigen is targeted to an antigen-presenting cell to enhance the processes of internalization and presentation by these cells. In one embodiment of the invention, a bispecific binding reagent is employed to target the

antigen to the cell. The bispecific binding agent specifically binds the antigen (either directly, to an epitope of the antigen or indirectly, to an epitope attached to the antigen) and, at the same time, binds a surface receptor of an antigen-presenting cell which can internalize antigen for processing and presentation. The receptor-binding component of the bispecific binding agent (and thus the bispecific binding agent itself) binds the receptor of the antigen-presenting cell without substantially being blocked by the natural ligand for the receptor. As a result, targeting of the antigen to the receptor will not be prevented by physiological levels of the ligand and the targeted receptor will remain capable of binding the ligand and functioning.

The preferred surface receptors of antigen-presenting cells for targeting are the receptors for the Fc region of IgG (FcγR). These receptors can mediate internalization of antibody-complexed antigens. The most preferred target is the high affinity Fc receptor (FcγRI). As described in more detail below, the bispecific binding agents are generally made of antibodies, antibody fragments or analogues of antibodies containing antibody-derived, antigen-binding (variable) regions. Antibodies that bind to Fc receptors on antigen-presenting cells, and whose binding to the receptor is not blocked by the natural ligand, can be produced by conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein (1975) Nature 256:495. Although somatic cell hybridization procedures are preferred, in

principle, other techniques for producing monoclonal antibodies can be employed e.g., viral or oncogenic transformation of B lymphocytes.

In general, an animal is immunized with an FcγR-bearing cell, a receptor-bearing portion thereof or the Fc receptor molecule in purified or partially purified form. Antibodies are selected which bind an epitope of the FcγR which is located outside of the ligand (i.e., Fc) binding domain of the receptor. This binding is not inhibited by IgG and, in turn, does not inhibit the binding of IgG and the function of the Fc receptor.

The production and characterization of monoclonal antibodies which bind FcγRI without being blocked by human IgG are described by Fanger et al. in PCT application WO 88/00052 and in U.S. Patent No. 4,954,617, the teachings of which are incorporated by reference herein. These antibodies bind to an epitope of FcγRI which is distinct from the Fc binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mab 22, mab 32, mab 44, mab 62 and mab 197. The hybridoma producing mab 32 is available from the American Type Culture Collection, Rockville, MD, ATCC No. HB9469.

The bispecific binding agent for targeting the antigen can be a heteroantibody, a bispecific antibody or an analogue of either of these. Bispecific antibodies are single, divalent antibodies which have two different antigen binding sites (variable regions). In the bispecific antibodies of this invention, one of the antigen binding sites is

specific for the receptor of the antigen-presenting cell and has the characteristics set forth above, and the other binding site is specific for the antigen to be targeted to the antigen-presenting cell. These antibodies can be produced by chemical techniques (see e.g., Kranz, D.M. et al. (1981) Proc. Natl. Acad. Sci. USA 78:5807), by "polydoma" techniques (See U.S. Patent 4,474,893, to Reading) or by recombinant DNA techniques.

Heteroantibodies are two or more antibodies or antibody-binding fragments (Fv, Fab, Fab' or $F(ab')_2$) of different binding specificity linked together. Heteroantibodies comprise an antibody (or antigen-binding fragment) specific for the receptor of the antigen-presenting cell, coupled to an antibody (or antigen binding fragment) specific for the antigen to be targeted. Heteroantibodies can be prepared by conjugating together two or more antibodies or antibody fragments. Preferred heteroantibodies are comprised of crosslinked Fab fragments. A variety of coupling or crosslinking agents can be used to conjugate the antibodies. Examples are protein A, carboiimide, N-succinimidyl-S-acetyl-thioacetate (SATA) and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). See e.g., Karpovsky et al. (1984) J. Exp. Med. 160:1686; Liu, M.A. et al. (1985) Proc. Natl. Acad. Sci. USA 82:8648. Other methods include those described by Paulus, H. Behring Inst. Mitt., No. 78, 118-132 (1985); Brennan et al. (1985) Science 229:81-83 or Glennie et al. (1987) J. Immunol. 139:2367-2375.

Bispecific binding agents can also be prepared from single chain antibodies. See e.g., Huston, J.S. et al. (1988) Proc. Natl. Acad. Sci. 85:5879; Skerra, A. and Plucthun, A. (1988) Science 240:1038. These are analogues of antibody variable regions produced as a single polypeptide chain. To form the bispecific binding agent, the single chain antibodies may be coupled together chemically or by genetic engineering methods.

As used herein, the term antigen means any natural or synthetic antigenic substance, a fragment or portion of an antigenic substance, a peptidic epitope, or a hapten. Suitable antibodies against wide variety of antigens for construction of the bispecific binding agents are available or can be readily made by standard techniques.

In some cases, it may be desirable to couple a substance which is weakly antigenic or nonantigenic in its own right (such as a hapten) to a carrier molecule, such as a large immunogenic protein (e.g., a bacterial toxin) for administration. In these instances, the bispecific binding reagent can be made to bind an epitope of the carrier to which the substance is coupled, rather than an epitope of the substance itself.

In another embodiment of the invention, the antigen can be coupled directly to the binding agent for the receptor. For example, an antigen can be coupled to an antibody, or fragment thereof, specific for an Fc receptor of an antigen-presenting cell. Proteinaceous antigens can be coupled by the methods described above or by other methods.

The antigen targeted by the method of this invention can be soluble or particulate; it may carry B cell epitopes, T cell epitopes or both. The antigen can be bacterial, viral or parasitic in origin. Often, the antigen will comprise a component of the surface structure of a pathogenic organism. For example, the antigen can comprise a viral surface structure such as an envelope glycoprotein of human immunodeficiency virus (HIV) or the surface antigen of hepatitis virus. In addition, the antigen can be associated with a diseased cell, such as a tumor cell, against which an immune response may be raised for treatment of the disease. The antigen can comprise a tumor-specific or tumor-associated antigen, such as the Her-2/neu proto-oncogene product which is expressd on human breast and ovarian cancer cells (Slamon, D.J. et al. (1989) Science 244:707).

Targeted immunostimulation can be performed in vitro or in vivo. The bispecific binding agent can be used to target an antigen to antigen-presenting cells in culture. Immunocompetent cells are separated and purified from patient blood. The cells are exposed to the antigen and the binding agent. Targeted antigen-presenting cells will process the antigen and present fragments on their surface. After stimulation, the cells can be returned to the patient.

To elicit an immune response in vivo, the antigen can be administered to a host in conjunction with the binding agent. Although in some circumstances the two may be administered separately, typically, they are administered as a preformed immunochemical complex. The complex is formed by

incubating the antigen and the bispecific binding agent at a desired molar ratio under conditions which permit binding of the two species. For example, the antigen and the bispecific binding reagent can be incubated in saline solution at 37°C. In some embodiments, for therapy of a pre-existing condition, the bispecific binding agent may be given without accompanying antigen.

The complex is administered in a physiologically acceptable solution at a dosage which will evoke an immune response against the antigen. The optimum dose of antigen, as well as the molar ratio of antigen and binding agent, may vary dependent upon factors such as the type of antigen, the immune status of the host, the type of infection or other disease being treated, etc. In most cases, the dose of antigen required to elicit an immune response (as determined by any standard method for assessment of immune response) should be lower than that which would be required if the antigen were given alone or as a complex with a monospecific antibody for the antigen.

The method of this invention can be used to enhance or reinforce the immune response to an antigen. For example, the method is valuable for the treatment of chronic infections, such as hepatitis and AIDS, where the unaided immune system is unable to overcome the infection. It can also be used in the treatment of the acute stages of infection when reinforcement of immune response against the invading organism may be necessary.

The method can be used to reduce the dose of antigen required to obtain a protective or therapeutic immune response or in instances when the host does not respond or responds minimally to the antigen. Although generally desirable, the lowering of effective dose can be especially desirable when the antigen is toxic to the host.

The method of targeted immunostimulation can also be used in disease therapy. For example, the bispecific binding agent can be used to target a tumor-associated (or tumor-specific) antigen to an antigen-presenting cell in order to cause or to enhance an immune response against the tumor.

The invention is illustrated further by the following exemplification:

Exemplification

Example 1

A bispecific heteroantibody was prepared from a monoclonal antibody against human erythrocytes (mono-D, a human anti-RhD antibody) and anti-FcγRI antibody 32, by a protocol previously described. Shen, C. et al. (1986) J. Immunol. 137:3378. Human erythrocytes were washed three times in buffer solution and then incubated for 60 minutes at 37°C in solution of the heteroantibody. After the incubation and three washings, erythrocytes coated with heteroantibody were diluted at $5x10^7$ cells per millimeter in Hank's buffer and then incubated with adherent monocytes (macrophages) at the ratio of 100:1 for one hour at 37°C. Cells were then washed in phosphate buffered saline (PBS), fixed for one

minute in ethanol and stained with Giemsa for observation through a light microscope.

Internalization of erythrocytes was easily observed as unstained spheres in the macrophage cytoplasm. The number of macrophages that internalized at least one erythrocyte were counted. This experiment was repeated numerous times with and without the heteroantibody present. In each experiment, no erythrocyte internalization was observed in macrophages which were incubated with erythrocytes in the absence of the heteroantibody.

In addition, experiments were performed after treatment of adherent monocytes (macrophages) with various concentrations of interferon-gamma which is known to increase the number of FcγRI receptors on the macrophage surface. Petroni, K.C. et al. (1988) J. Immunol. 140:3467. As shown in the table below, the number of macrophages that internalized erythrocytes increased in a direct relation to the concentration of interferon-gamma.

Table

Gamma Interferon Concentration (µg/ml)	Percentage of Macrophages Having Internalized at Least One Erythrocyte (%)
1000	40
100	25
10	6

These data show that the heteroantibody can trigger internalization of antigen by macrophages.

Example 2 Enhanced Tetanus Toxoid (TT) presentation by directing TT to human FcγR.

Monoclonal antibody 22 (mAb 22) is specific for the high affinity Fcγ receptor and its binding to the receptor is not blocked by IgG Fc. See U.S. Patent No. 4,954,617. TT was conjugated to $F(ab')_2$ of mAb 22. To test the potential role of human antibody (Ab) isotype, TT was conjugated to non-specific $HIgG^1$. TT (obtained from Accurate Chemical Co., Westburg, NY) was linked to antibody or antibody fragments by the SATA-malemide procedure.

The experiments were done in serum free AIM V medium (Gibco, Grand Island, NY) to minimize the contribution of undefined components such as hormones, lymphokines or monomeric and polymeric immunoglobulins. The use of AIM V reduces non-specific T cell responses while maintaining Ag-specific responses equal to those observed with other media tested. This medium allows more definitive studies of Fc receptor-enhanced antigen presentation in vitro. If antigen is directed to Fc receptors using mAb that bind to Fc receptors regardless of the presence of human IgG, this medium is not a requirement to see enhanced Ag presentation.

T cells used in the assay were primed with TT. When T cells are taken fresh from an individual there are T cells present which can potentially respond to many things (serum components, mouse Ig, etc.). By priming the cells in vitro (i.e., adding TT to fresh monocytes and T cells), only the T cells which recognize TT grow out. Thus, the cells are specific for TT.

The T cells were taken from the same donor as the monocytes. The vast majority (>85%) are CD4+, helper T cells specific for TT. They are polyclonal which means they likely recognize many parts of TT (i.e., many different 10-20 amino acid segments of TT as foreign). This is the type of response (polyclonal) which one might expect in vivo.

5×10^4 monocytes purified by cold aggregation and 5×10^4 T cells (primed once with TT, as described) were added in AIM V medium to wells of a 96 well plate. Subsequently, Ab, TT, TT-Ab, or anti-TT Ab + TT was added. Plates were incubated 72 hrs at 37°C at which time [^{3}H]thymidine was added overnight. Cells were then harvested and counted.

Figure 1 shows the results of these experiments. Data is expressed as counts/minute (CPM) ± SD. As can be seen, TT conjugated to mAb 22 resulted in enhanced T cell proliferation over that obtained with TT alone, HIgG1-TT or anti-TT:TT complex. Ab alone did not induce T cell proliferation.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

1. A method of stimulating an immune response to an antigen, comprising administering the antigen and a binding agent which binds a surface receptor of an antigen-presenting cell without being blocked substantially by the natural ligand for the receptor, so that the antigen is targeted to the receptor.

2. A method of claim 1, wherein the antigen is coupled to the binding agent.

3. A method of claim 2, wherein the binding agent is an antibody, or fragment thereof.

4. A method of claim 1, wherein the binding agent is bispecific, having a binding affinity for the receptor and for the antigens.

5. A method of claim 4, wherein the antigen and the bispecific binding agent are administered as a complex.

6. A method of claim 4, wherein the bispecific binding agent is a heteroantibody.

7. A method of claim 1, wherein the antigen is selected from the group consisting of viral, bacterial, parasite and tumor-associated antigen.

8. A method of claim 1, wherein the antigen is derived from hepatitis virus.

9. A method of claim 1, wherein the hepatitis antigen is hepatitis surface antigen.

10. A method of claim 1, wherein the antigen is an HIV antigen.

11. A method of claim 1, wherein the antigen-presenting cell is a macrophage.

12. A method of claim 1, wherein the surface receptor of the macrophage is a receptor for immunoglobulin Fc.

13. A method of claim 12, wherein the receptor for immunoglobulin Fc is the high affinity Fc receptor for immunoglobulin G.

14. A method of stimulating an immune response against an antigen, comprising administering a molecular complex comprising an antigen and a bispecific heteroantibody, the heteroantibody comprising a first antibody, or fragment thereof, which specifically binds the Fc receptor for immunoglobulin G (IgG) on the macrophage surface without being blocked substantially by IgG and a second antibody, or fragment thereof, which specifically binds the antigen.

15. A method of claim 14, wherein the bispecific antibody comprises a Fab x Fab conjugate.

16. A method of treating hepatitis B infection comprising administering to an individual infected with the virus a molecular complex comprising hepatitis B surface antigen, or portion thereof, and a Fab x Fab heteroantibody wherein the first Fab binds the high affinity Fc receptor for immunoglobulin G without being blocked substantially by IgG and the second Fab binds the antigen.

17. A method of stimulating an immune response to an antigen, comprising administering a complex of the antigen coupled to a binding agent which binds an antigen-presenting cell without being blocked substantially by the natural ligand for the receptor.

18. A method of claim 17, wherein the surface receptor of the macrophage is a receptor for immunoglobulin Fc.

19. A molecular complex comprising an antigen complexed to a bispecific binding agent which binds a surface receptor of an antigen-presenting cell without being blocked substantially by the natural ligand for the receptor and binds the antigen.

20. A molecular complex of claim 19, wherein the bispecific binding agent is a heteroantibody.

21. A molecular complex of claim 20, wherein the heteroantibody comprises chemically crosslinked Fab or Fab' antibody fragments.

22. A molecular complex of claim 19, wherein the antigen is selected from the group consisting of viral, bacterial, parasite and tumor-associated antigen.

23. A molecular complex of claim 19, wherein the antigen is a hepatitis antigen.

24. A molecular complex of claim 19, wherein the antigen is an HIV antigen.

25. A molecular complex of claim 19, wherein the antigen-presenting cell is a macrophage.

26. A molecular complex of claim 25, wherein the surface component of the macrophage is a receptor for immunoglobulin Fc.

27. A molecular complex of claim 26, wherein the receptor for immunoglobulin Fc is the high affinity Fc receptor for immunoglobulin G.

28. A molecular complex, comprising an antigen and a bispecific heteroantibody, the heteroantibody comprising a first antibody, or fragment thereof, which specifically binds an Fc receptor for immunoglobulin G (IgG) on the macrophage surface without being blocked substantially by IgG and a second antibody, or fragment thereof, which specifically binds the antigen.

29. A molecular complex of claim 28, wherein the first antibody, or fragment thereof, binds the high affinity Fc receptor for IgG.

30. A molecular complex of claim 28, wherein the antigen is selected from the group consisting of a viral, bacterial, parasitic and disease-associated antigen.

31. A molecular complex of claim 28, wherein the antigen is a hepatitis antigen.

32. A molecular complex of claim 25, wherein the hepatitis antigen is hepatitis surface antigen.

33. A molecular complex of claim 26, wherein the antigen is an HIV antigen.

34. A molecular complex, comprising an antigen and a Fab x Fab heteroantibody, wherein the first Fab binds the high affinity Fc receptor for immunoglobulin G (IgG) without being blocked by IgG and the second Fab binds the antigen.

35. A vaccine composition, comprising a molecular complex of claim 19 in a pharmaceutically acceptable vehicle.

36. An antigen linked to an antibody, a fragment or analogue thereof, which binds the Fcγ receptor of an antigen-presenting cell without being blocked by IgG Fc.

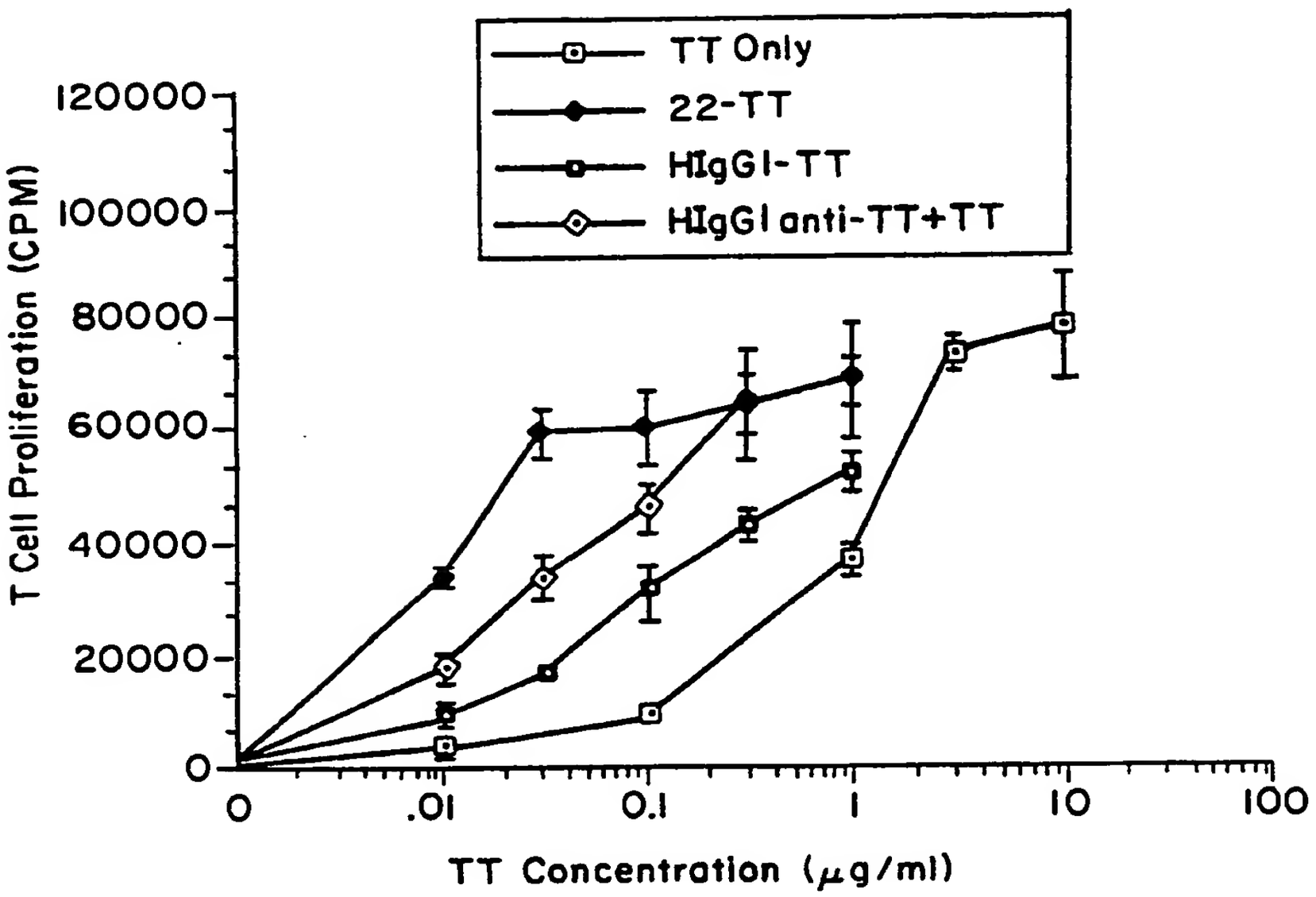
TT Only
22-TT
HIgG1-TT
HIgG1anti-TT+TT
120000
100000
80000
60000
40000
20000
0
T Cell Proliferation (CPM)
0
.01
0.1
1
10
100
TT Concentration (μg/ml)

FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07283

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 35/16, 35/26, 37/04; C12N 5/02

USCL.: 424/85.8, 88; 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
USCL.	424/85.8, 88; 530/387

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	U.S.A. 4,950,480 (Barber et al.) 21 August 1990. see abstract. summary of invention. Examples I-III.	1-7,11-20 21-36
X Y	WO.A. 88/00052 (Fanger et al.) 14 January see entire documemnt.	1-36 1-36

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

20 January 1992

Date of Mailing of this International Search Report

10 FEB 1992

International Searching Authority

ISA/USA

Signature of Authorized Officer

Lila Feisee

Form PCT/ISA/210 (second sheet) (Rev.11-87)